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Relation of changes in amount and type of dietary fat to fecapentaenes in premenopausal women

Philip R. Taylor¹, Mark H. Schiffman², D. Yvonne Jones¹, Joseph Judd³,
Arthur Schatzkin¹, P.P. Nair³, Roger Van Tassell⁴ and Gladys Block⁵

¹ Cancer Prevention Studies Branch, Division of Cancer Prevention and Control, NCI, Bethesda, MD 20892,

² Environmental Epidemiology Branch, Division of Cancer Etiology, NCI, Bethesda, MD 20892.

³ Lipid Nutrition Laboratory, Beltsville Human Nutrition Research Center, Agriculture Research Center, USDA, Beltsville, MD 20705,

⁴ Department of Anaerobic Microbiology, Virginia Tech, Blacksburg, VA 24061,

and ⁵ Surveillance and Operations Research Branch, Division of Cancer Prevention and Control, NCI, Bethesda, MD 20892 (U.S.A.)

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Summary

Correlation studies suggest that fecal mutagenicity is increased in groups eating high-fat diets, the same groups who are often found to have high colorectal cancer incidence and mortality. The fecapentaenes are the best characterized class of fecal mutagens, but the relationship of dietary fat intake to the excretion of these potent genotoxins is unknown. We studied the effect of changes in amount and type of dietary fat on fecapentaene levels in 31 premenopausal women 20–40 years of age who participated in a controlled feeding study. After a pre-diet free-living period lasting 1 menstrual cycle, women were placed on a high-fat (40% energy from fat) diet for 4 menstrual cycles and then switched to a low-fat (20% energy from fat) diet for an additional 4 menstrual cycles. One-half the subjects were maintained throughout the study at a ratio of polyunsaturated-to-saturated fatty acids (*P/S* ratio) of 1.0, the other half at 0.3; body weight was constant. All meals during the controlled diet periods were prepared at the Human Study Facility of the Beltsville Human Nutrition Research Center. Fecapentaene and fecapentaene precursor levels were measured in acetone extracts from 3-day pooled stool samples collected during the study. No differences in fecapentaene or precursor levels were observed between the high- and low-fat diets at either *P/S* ratio. Fecapentaene and precursor levels were higher while on controlled diets than during the pre-diet free-living period, and levels declined again in the post-diet free-living period. We conclude that dietary fat has no significant effect on fecapentaene or precursor levels in acetone extracts of stool in premenopausal women. The effect of other dietary or non-dietary factors on fecapentaenes remains unknown.

Correspondence: Dr. Philip R. Taylor, Cancer Prevention Studies Branch, Division of Cancer Prevention and Control, NCI, Bethesda, MD 20892 (U.S.A.).

The fecapentaenes are newly characterized, potent mutagens excreted in the feces of humans and some animals (Gupta et al., 1983; Hirai et al.,

1982; Pertel and Sellers, 1986). These ether-linked lipids are highly genotoxic in the Ames Salmonella assay and cause DNA damage and mutations in human cells as well (Plummer et al., 1986; Goggelmann et al., 1986; Curren et al., 1987). Since mutagenicity is commonly used to screen for possible carcinogenicity, the discovery of this class of fecal mutagens has raised hopes of identifying fecal carcinogens that cause colorectal cancer. However, the origins, determinants, and in vivo effects of the fecapentaenes are still poorly understood.

As a result, the fecapentaenes are now under investigation by researchers in several disciplines. Microbiologists are trying to identify the metabolic precursor of the fecapentaenes, which are known to be produced by colonic *Bacteroides* species from an unidentified substrate (Van Tassell and Wilkens, 1985). They also hope to determine which colonic factors cause certain individuals to excrete consistently high fecapentaene concentrations while others produce no detectable stool levels (Van Tassell et al., 1986). Biochemists are studying the unusual instability of the fecapentaenes, which degrade rapidly and unpredictably when exposed to oxygen (Goggelmann et al., 1986). Molecular biologists are exploring the interactions of the fecapentaene molecule with human DNA (Gupta et al., 1984). Carcinogenicity testing is currently under way. Epidemiologists are examined the influences of factors such as age, race, sex, and diet on excreted fecapentaene concentrations in healthy populations in comparison to patients with colorectal cancer (Schiffman, 1986). The influence of diet on fecapentaene levels is of special interest. Increased fecal mutagenicity has been observed in populations eating high-fat and low-fiber diets, the same groups known to be at increased risk of colorectal cancer (Correa and Haenszel, 1978; Ehrlich et al., 1979; Mower et al., 1982; Reddy et al., 1980). Hypothetically, a high-fat or low-fiber diet could increase colorectal cancer risk by elevating the total concentration of genotoxic compounds, such as the fecapentaenes, in the colon (Goldin, 1980; Reddy et al., 1987).

The dietary study described here was used to test the corollary hypothesis that a low-fat diet could decrease the levels of one specific class of genotoxins, the fecapentaenes, in the fecal stream.

Since the concentrations of metabolic precursors to the fecapentaenes may relate more directly to diet than do fecapentaene levels themselves, an indirect measurement of metabolic precursors to the fecapentaenes was also made.

Methods

Premenopausal women 20–40 years of age were recruited from the Beltsville, Maryland area to study the effects of eating high-fat (40% energy from fat) versus low-fat (20% energy from fat) diets at low (0.3) or high (1.0) ratios of polyunsaturated-to-saturated fatty acids (*P/S* ratios). The dietary trial was used to assess multiple biologic parameters, including fecapentaene excretion. As a result, women were not selected on the basis of fecapentaene measurements. 97 women responded to local advertisements; 40 of the volunteers were considered eligible based on a prescreen questionnaire (no history of metabolic or chronic disease, no regular medications, no menstrual irregularities, not pregnant or lactating, no unusual dietary patterns); 37 passed a screening evaluation which included a physical examination and hematologic and biochemical blood tests, and started the study; and 31 completed the study. Subjects were paired on relative weight (weight/height) and randomized to one of two *P/S* groups.

After a pre-diet free-living period lasting 1 menstrual cycle, the women were then placed on high-fat diet for 4 menstrual cycles and the switched to the low-fat diet for a similar period of 4 menstrual cycles.

During the controlled dietary periods, all meals were prepared in the Human Study Facility of the Beltsville Human Nutrition Research Center (BHNRC). Breakfast and evening meals on weekdays were eaten in the BHNRC dining facility while carry-out meals were provided for weekday lunches and all weekend meals. A 14-day menu cycle was utilized, formulated from commonly available foods. Women started the study at the caloric level closest to their estimated maintenance requirement, with total caloric intake adjusted during the study to maintain body weight. The women were weighed each weekday while taking the controlled diets and were interviewed at that time by the study nurse who recorded any dietary

irregularities and stressed the importance of compliance.

Fecal samples were collected at the midpoint of the menstrual cycle 6 times during the study: pre diet (a 1-month free-living period prior to diets), during the second and fourth menstrual cycles of the high-fat diet, during the second and fourth menstrual cycles of the low-fat diet, and post diet (a free-living period approximately 4 months after completing the low-fat diet). All fecal samples were 3-day pooled specimens. Collections were made in plastic bags and immediately stored on dry ice after which specimens were homogenized and lyophilized, then re-stored at -40°C or colder until analysis. Since fecapentaene degradation during prolonged storage was a concern, sealed, lyophilized samples were assayed as soon as feasible, within a few months of the end of each collection period. A systematic subsample of specimens from all batches was re-analyzed at the end of the study to assess batch effects and to rule out laboratory drift. The conclusions were not affected, so only the original measurements are presented here. Fecapentaene assays were performed in a masked fashion at the Department of Anaerobic Microbiology at Virginia Tech. Two measurements were made for each sample. Total fecapentaenes were quantified in acetone extracts of the lyophilized stool by high-performance liquid chromatography, following previously published methods (Van Tassell et al., 1986). Also measured were the total fecapentaenes in lyophilized samples incubated anaerobically in nutrient broth, with added *Bacteroides thetaiotaomicron*, for 96 h. The conditions of incubation were designed to promote maximum production of fecapentaenes from available precursors in the samples (Van Tassell et al., 1986). Thus, the incubated fecapentaene value can be viewed as a measurement of potential fecapentaene, or "precursor", concentration. All assay results were expressed in nanograms per gram of lyophilized stool (ng/g). Samples with values below the detection limit (20 ng/g) were given a value of 10 ng/g while values in the detectable but nonquantifiable range (20–100 ng/g) were given a value of 60 ng/g.

Dietary intake was assessed at baseline with 7-day diet records. Composite samples of duplicate menus were analyzed to determine precise

nutrient intake while on the controlled diets. A quantitative food frequency questionnaire (Block et al., 1986) was used to assess diet at the time of sample collection during the post-diet free-living period. An investigation was carried out (report in preparation) which indicated that the questionnaire estimates corresponded well with those produced by 7-day diet records.

Statistical analyses were carried out using the Statistical Analysis System (SAS) software. As the distribution of paired differences were typically not normal, even with transformation, analyses were limited to nonparametric methods. The Wilcoxon rank sum test was used to compare the unpaired differences between P/S groups, the Wilcoxon signed-rank test was used for all paired differences, and the correlation between measurements used the rank methods of Spearman.

Results

The empirical distribution functions for both fecapentaene and precursor values from the pre-diet free-living period are shown in Fig. 1. The median value for fecapentaenes was 252 ng/g (range 10–14 001) while the median value for precursor was 5451 (range 60–39 910). Spearman correlation coefficients between fecapentaene and precursor levels from the same sample within each of 6 study periods were consistent and high, ranging from 0.54 to 0.65.

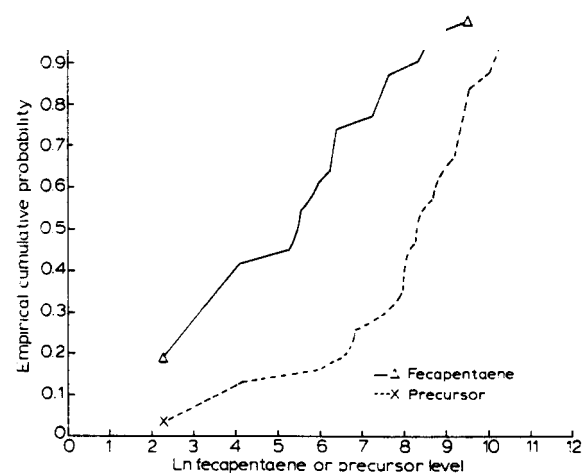


Fig. 1. Cumulative distribution function of pre-diet fecapentaene and precursor levels.

TABLE 1

MEAN (S.E.M.) DAILY NUTRIENT INTAKE FOR SUBJECTS FOR THE 4 STUDY PERIODS

	Study period				
	High-fat <i>P/S</i> = 0.3 (<i>N</i> = 15)	High-fat <i>P/S</i> = 1.0 (<i>N</i> = 16)	Low-fat <i>P/S</i> = 0.3 (<i>N</i> = 15)	Low-fat <i>P/S</i> = 1.0 (<i>N</i> = 16)	Post diet (<i>N</i> = 17) ^b
Energy (kcal)	2278 (65)	2180 (81)	2260 (97)	2280 (110)	1841 (189)
Protein (% kcal)	16	16	17	17	17
Carbohydrate (% kcal)	45	45	64	64	46
Fat (% kcal)	39	39	19	19	37
Cholesterol (mg)	374 (11)	289 (10)	230 (9)	199 (9)	270 (32)
Saturated fat (g)	44.2 (1.3)	26.8 (1.1)	20.9 (0.7)	12.3 (0.6)	26.2 (3.9)
Oleic acid (g)	30.5 (0.9)	33.5 (1.4)	14.9 (0.6)	17.0 (0.8)	25.5 (3.0)
Linoleic acid (g)	14.6 (0.4)	26.1 (0.9)	6.9 (0.2)	12.9 (0.6)	13.0 (2.4)
Crude fiber (g)	5.8 (0.2)	5.6 (0.2)	7.9 (0.2)	7.7 (0.3)	— ^c

^a From 7-day records.^b Dietary values as estimated by Block Self-administered Diet History Questionnaire (Block et al., 1986).^c Crude fiber not estimated by this questionnaire.

Table 1 shows estimated dietary intake for study participants over the 4 dietary periods of the study.

For both fecapentaenes and precursor, values were similar across *P/S* groups at each of the 6 sample periods except for fecapentaene levels at the end of the high-fat period where the *P/S* = 1.0 group was much lower than the *P/S* = 0.3 group (median values 173 and 2040 ng/g, respectively; data not shown). These two *P/S* groups were pooled for all remaining analyses.

Table 2 shows the median and range for fecapentaene and precursor levels for all 6 study periods. The values increased in going from the pre-diet to the high-fat diets, rose slightly on the low-fat diet, and then fell when subjects returned to their post-study free-living diet.

The remainder of the analyses were conducted in a pairwise fashion (i.e., each woman compared to herself). There were no differences in fecapentaene or precursor values between the midpoint and end of the high-fat period (for fecapentaene, *p* = 0.793; for precursor, *p* = 0.742). Similarly, there were no differences between the midpoint and end of the low-fat period (for fecapentaene, *p* = 0.127; for precursor, *p* = 0.333). Consequently the two high-fat values were averaged for subsequent analysis and presentation as were the two low-fat values.

The distributions of the paired differences in fecapentaene levels between baseline and the 3 other dietary periods for the study are shown in Fig. 2. The same picture was observed for precursor levels (data not shown). No difference in the distribution of differences was observed between the high- and low-fat periods for fecapentaenes (median increase from high- to low-fat periods = 591 ng/g stool, *p* = 0.051) or precursor

TABLE 2

FECAPENTAENE (AND PRECURSOR) LEVELS BY STUDY PERIOD (ng/g dry weight stool)

Study period	Number	Median	Range
Middle of high-fat			
End of high-fat			
Middle of low-fat			
End of low-fat			
Post diet			

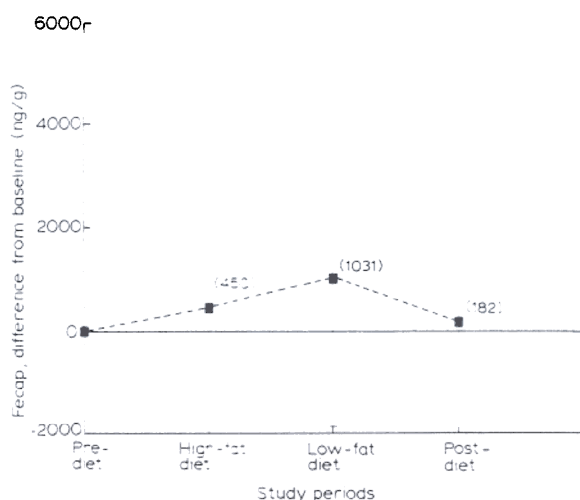


Fig. 2. Fecapentaene levels (difference from baseline). Median, 10th and 90th percentiles of distribution.

levels (median increase = 474 ng/g stool, $p = 0.564$).

Fig. 3 shows individual plots of fecapentaene values for the controlled high- and low-fat diet periods.

Finally, because fecapentaene and precursor levels appeared to be higher during the controlled diets than in both the pre- and post-diet periods, we compared pre-diet to controlled diet (an average of all 4 values obtained during the controlled diet periods) and controlled diet to post-diet. For fecapentaene values, the median difference among individuals going on study was an increase of 1085 ng/g stool ($p = 0.003$), while the median difference in going off study was a drop of 840 ng/g stool ($p = 0.097$). Results for precursor values followed a similar pattern (median on-study increase = 7471 ng/g stool, $p = 0.0004$; median off-study decrease = 4087 ng/g stool, $p = 0.037$).

Discussion

We studied the change in levels of fecapentaenes and their metabolic precursors in acetone-based fecal extracts from premenopausal women, in response to a tightly monitored reduction of dietary fat. We had hypothesized that a low-fat diet would lower fecapentaene values, based on two previous observations: (1) groups

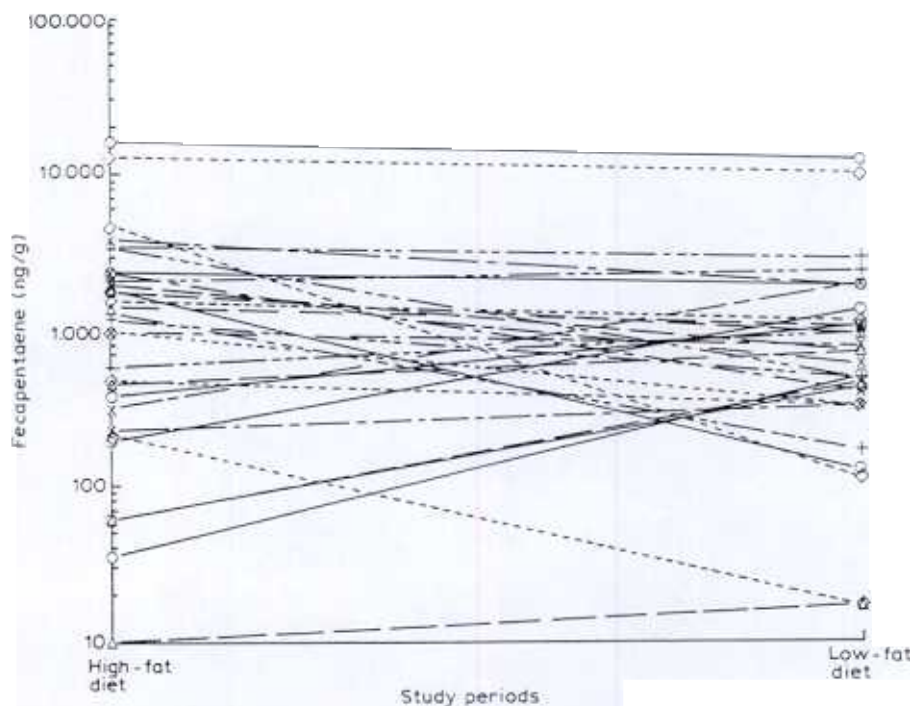


Fig. 3. Fecapentaene values for high- and low-fat periods of study

with low-fat, high-fiber diets have been shown in previous correlational studies to excrete lower total mutagenicity than groups eating Western-style diets (Ehrich et al., 1979; Reddy et al., 1980); and (2) fecapentaenes account for much of the mutagenicity of acetone-based fecal extracts (Dion and Bruce, 1983). However, both fecapentaene and precursor levels increased between high- and low-fat diets, though these differences were not statistically significant. The only effects that were significant related to being "on study". Both fecapentaene and precursor levels increased markedly after starting the controlled diet, and both decreased after going off study. This finding suggests that dietary factors apart from fat may influence fecapentaene levels, but the variables responsible for the study effect are unknown.

While other studies have reported changes in fecal mutagenicity in response to dietary manipulation (Reddy et al., 1987; Bruce and Dion, 1980; de Vet et al., 1981; Dion et al., 1982; Kuhnlein and Kuhnlein, 1980; Kuhnlein et al., 1983), this is the first report of fecapentaene measurements following controlled dietary changes. If elevated dietary fat is ultimately shown to increase the risk of colorectal cancer, it appears based on our investigation that this effect is not mediated by the concentration of genotoxic fecapentaenes in the colon. Our results corroborate a mutagenicity study by Bruce and Dion (1980), in which dietary fat supplementation had no effect on the mutagenicity of organic-based fecal extracts from 9 subjects. Their genotoxicity assay was *Salmonella* tester strain TA100 without S9 activation, a method known to detect the mutagenic effect of the fecapentaenes. However, other genotoxins detected using different laboratory techniques may have varying dietary determinants. For example, Kuhnlein et al. (1983) observed an increase in water-soluble fecal mutagenicity following an increase in dietary fat plus refined grains, in their trial of 6 subjects.

Thus, our results must be interpreted with caution since they do not exclude a potential reduction in the levels of fecal mutagens other than the fecapentaenes in response to dietary fat reduction. This possibility is currently under study using the same stool specimens as we tested for the current presentation. Moreover, the effects of other di-

etary manipulations and the influence of non-dietary factors on fecapentaene excretion are unknown and merit further study.

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References

- Block, G., A.M. Hartman, C.M. Dresser, et al. (1986) A data-based approach to diet questionnaire design and testing, *Am. J. Epidemiol.*, 124, 453-469.
- Bruce, W.R., and P.W. Dion (1980) Studies relating to a fecal mutagen, *Am. J. Clin. Nutr.*, 14, 79-85.
- Correa, P., and W. Haenszel (1978) The epidemiology of large-bowel cancer, *Adv. Cancer Res.*, 26, 1-141.
- Curren, R.D., D.L. Putnam, L.L. Yang, et al. (1987) Genotoxicity of fecapentaene-12 in bacterial and mammalian cell assay systems, *Carcinogenesis*, 8(2), 349-352.
- de Vet, H.L., C. Sharma and B.S. Reddy (1981) Effect of dietary fried meat on fecal mutagenic and co-mutagenic activity in humans, *Nutr. Rep. Int.*, 23, 653-660.
- Dion, P.W., E.B. Bright-See, C.C. Smith, et al. (1982) The effect of dietary ascorbic acid and alpha-tocopherol on fecal mutagenicity, *Mutation Res.*, 97, 267-281.
- Ehrich, M., J.E. Aswell, R.L. Van Tassell, et al. (1979) Mutagens in the feces of 3 South African populations at different levels of risk of colon cancer, *Mutation Res.*, 64, 231-240.
- Goggelmann, W., F.K. Maier and H.R. Pfaendler (1986) Mutagenicity of synthetic racemic fecapentaene-12, *Mutation Res.*, 174(3), 165-167.
- Goldin, B. (1980) The role of diet and intestinal flora in the etiology of large bowel cancer, in: S. Winawer, D. Schottenfeld and P. Sherlock (Eds.), *Colorectal Cancer: Prevention, Epidemiology, and Screening*, Raven, New York, pp. 43-50.
- Gupta, I., J. Baptista, W.R. Bruce, et al. (1983) Structures of fecapentaenes, the mutagens of bacterial origin isolated from human feces, *Biochemistry*, 22, 241-245.
- Gupta, I., K. Suzuki, R.W. Bruce, et al. (1984) A model study of fecapentaenes: mutagens of bacterial origin with alkylating properties, *Science*, 225(4661), 521-523.
- Hirai, N., D.G.L. Kingston, R.L. Van Tassell, et al. (1982) Structure elucidation of a potent mutagen from human feces, *J. Am. Chem. Soc.*, 104, 6149-6150.
- Kuhnlein, H.V., and U. Kuhnlein (1980) Mutagens in feces from subjects on controlled formula diets, *Nutr. Cancer*, 2, 119-124.
- Kuhnlein, H.V., U. Kuhnlein and P.A. Bell (1983) The effect of short-term dietary modification on human fecal mutagenic activity, *Mutation Res.*, 113, 1-12.
- Mower, H.F., D. Ichinotsubo, L.W. Wang et al. (1982) Fecal mutagens in two Japanese populations with different colon cancer risks, *Cancer Res.*, 42, 1164-1169.

- Pertel, R., and R.L. Sellers Jr (1986) Production of fecal mutagens in the pig model for host/diet interactions (meeting abstract), *Fed. Proc.*, 45(4), 970.
- Plummer, S.M., R.C. Grafstrom, L.L. Yang, et al. (1986) Fecapentaene-12 causes DNA damage and mutations in human cells, *Carcinogenesis*, 7(9), 1607-1609.
- Reddy, B.S., C. Sharma, L. Darby, et al. (1980) Metabolic epidemiology of large bowel cancer: fecal mutagens in high- and low-risk populations for colon cancer: a preliminary report, *Mutation Res.*, 72, 511-522.
- Reddy, B.S., C. Sharma, B. Simi, et al. (1987) Metabolic epidemiology of colon cancer: effect of dietary fiber on fecal mutagens and bile acids in healthy subjects, *Cancer Res.*, 47, 644-648.
- Schiffman, M. (1986) Epidemiology of fecal mutagenicity, *Epidemiol. Rev.*, 8, 92-105.
- Van Tassell, R.L., and T.D. Wilkens (1985) The precursors of fecapentaenes — a preliminary report, *Proceedings of the International Meeting, "Intestinal Flora in Health and Disease"*, October 16-18, 1985, Rome, Italy.
- Van Tassell, R.L., R.M. Schram and T.D. Wilkins (1986) Microbial biosynthesis of fecapentaenes, in: J. Knudsen (Ed.), *Genetic Toxicology of the Diet*, Liss, New York, pp. 199-211.